REMARKS

The remainder of this Reply is set forth under appropriate sub-headings for the convenience of the Examiner.

Information Disclosure Statements

Applicants hereby bring to the attention of the Examiner the fact that the PTO Form 1449 associated with an Information Disclosure Statements filed on January 22, 2002 (references AA-AJ, AL, AM, AR-AZ, AR2-AZ2, AR3-AY3) and a transmittal of a Sixth Supplemental Information Disclosure Statement filed March 4, 2003 (US Patent Application No.: 10/251,685, filed September 20, 2002) were not returned by the Examiner in the Office Action. The Office Action included Forms PTO 1449 associated with Information Disclosure Statements filed on February 20, 2002, May 17, 2002, November 1, 2002, January 9, 2003 and February 5, 2003. For the Examiner's convenience, a copy of the Sixth Information Disclosure Statement and date-stamped postcard receipt, filed by Certificate of Mailing on March 4, 2003; and the Information Disclosure Statement, PTO Form 1449 and date-stamped postcard receipt, filed by Certificate of Mailing January 22, 2002, are enclosed with this Reply.

Applicants' Attorney spoke to Examiner Afremova on November 7, 2003 regarding the absence of reference to and an initialed PTO Form 1449 for the Information Disclosure Statement filed on January 22, 2002. Examiner Afremova indicated that, according to the Patent Office records, the January 22, 2002 Information Disclosure Statement and accompanying PTO Form 1449 have not been entered. As requested by Examiner Afremova, Applicants are filing a Transmittal of Previously Filed Information Disclosure Statement, a copy of the January 22, 2002 Information Disclosure Statement, PTO Form 1449, date-stamped postcard receipt and the references (AA-AJ, AL, AM, AR-AZ, AR2-AZ2, AR3-AY3) previously filed in the January 22, 2002 Information Disclosure Statement.

In addition, Applicants bring to the Examiner's attention a Seventh Supplemental Information Disclosure Statement filed on August 7, 2003, after issuance of the outstanding Office Action. A copy of the Seventh Supplemental Information Disclosure Statement, PTO Form 1449 and date-stamped postcard receipt filed on August 7, 2003 is enclosed for the

Examiner's convenience. The only reference cited in the August 7, 2003 Seventh Supplemental Information Disclosure Statement (AS6) was cited in the PTO-892 Form by the Examiner in the Office Action.

Further, Applicants are filing concurrently with this Reply an Eighth Supplemental Information Disclosure Statement.

Applicants respectfully request consideration of the references cited in the Information Disclosure Statements filed on January 22, 2002, the non-published pending application cited in a Sixth Supplemental Information Disclosure Statement on March 4, 2003 and the accompanying Eighth Supplemental Information Disclosure Statement. Applicants request that the Examiner initial the appropriate PTO 1449 Forms, including the form for the Seventh Supplemental Information Disclosure Statement, and transmittal form and return the initialed forms with a subsequent Office Action.

Applicants' Invention

Applicants' invention, as set forth in Group II, now pending Claims 14, 19-21, 23, 25 and 26, as amended, is directed to an isolated cell population derived from bone marrow, wherein greater than about 91% of the cells in the cell population co-express CD49c and CD90 and wherein the cell population has a doubling time of less than about 30 hours. The isolated cell populations of Applicants' claimed invention, as amended, can differentiate into a pre-selected phenotype including a chondrocyte, an astrocyte, an oligodendrocyte, a neuron, an osteoclast, an osteocyte, an osteoblast, a cardiomyocyte, a pancreatic islet cell, a skeletal muscle cell, a smooth muscle cell, a hepatocyte and a retinal ganglial cell. The isolated cell population of the Applicants' claimed invention, as amended, expresses p21 or p53 after between about 20 to about 50 population doublings of the cells. The isolated cell population of Applicants' claimed invention, as amended, can express at least one trophic factor selected from the group consisting of BDNF, IL-6, NGF and MCP-1. In all instances, as set forth, for example, on page 29, line 25 through page 30, line 2, the isolated cell population is a population of cells that uniformly retains a characteristic morphology of dividing cells without evidence of aged or terminally-differentiated cells.

Advantages of Applicants' Invention

Applicants' claimed isolated cell populations have many advantages. For example, Applicants' claimed isolated cell population maintains a phenotype characteristic of proliferative cells not of aged cells. The isolated cell populations of Applicants' claimed invention, as amended, can be employed in transplantation to treat a degenerative disease, an acute injury condition or a neurological condition as described, for example, on page 16, line 12 through page 17, line 27 of the specification. Applicants' claimed cell population can differentiate into a pre-selected phenotype following treatment of a mammal. For example, Applicants' isolated cell population can be used to treat spinal cord injury as shown and described in the specification, for example, on page 33, line 24 through page 35, line 2 and Figure 5.

Other advantages of Applicants' claimed invention include the generation of a homogeneous cell population which can be stored, propagated and used repeatedly to treat a mammal having a degenerative disease, acute injury condition or neurological condition. Applicants' claimed cell population, as amended, provides a consistent source of cells capable of differentiating into a preselected phenotype.

Amendments to the Claims

Claims 14, 19-21, 23, 25 and 26 have been amended to more clearly define Applicants' invention. Support for amendments to the claims can be found in the specification. For example, page 10, lines 17-18, describe a cell population of Applicants' invention as derived from bone marrow. Page 28, lines 5-6 and Figure 2B describe a cell population of Applicants' invention as a population wherein greater than about 91% of the cells are CD90 and CD49c positive. Page 29, lines 25-27 and Figure 4 describe an isolated cell population of Applicants' invention having a doubling rate of less than about 30 hours. Amendments to the claims do not introduce new matter. Entry is requested.

Claim Objections

Claims 14-26 were rejected because of informalities. In particular, the Examiner stated that Claim 14 appeared to have a typographical error in the word "which." The Examiner further

stated that Claim 21 appeared to have a typographical error using the capital letter "P" for p21 and p53.

Applicants have amended Claims 14 and 21 to obviate the claim objections.

Rejections of Claims 14-26 Under 35 USC § 112, Second Paragraph

Claims 14-26 are rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Examiner stated that Claim 14 was indefinite with respect to the negative expression of bone sialoprotein. The Examiner further stated that Claim 26 was indefinite since it was uncertain as to whether the cells claimed in Claim 26 were capable of expressing the cytokines under appropriate conditions or whether they were expressing the cytokines under the same conditions.

Applicants have amended Claims 14 and 26 to obviate the rejection. Specifically, Claim 14 has been amended to omit the negative limitation that the cells do not express bone sialoprotein. Claim 26 has been amended to more clearly indicate that the cells of Claim 26 further include trophic factors.

Rejection of Claims 14-26 Under 35 USC § 101

Claims 14-26 were rejected under 35 USC § 101 as not sufficiently distinguishing the claimed cells from cells that exist in nature. The Examiner further stated that the phrase "a substantially homogeneous cell population" did not particularly point out any non-naturally occurring differences between the claimed products and the naturally occurring bone marrow cell populations from which the isolated cell populations were derived.

As suggested by the Examiner, Applicants have amended the pending claims to include the term "isolated." Applicants have further amended the pending claims to clearly state that greater than 91% of the cells of the isolated cell population co-express CD49c and CD90. Amendments to the claims particularly point out the characteristics of Applicants' isolated cell population that distinguish the claimed invention from naturally occurring bone marrow cell populations. Therefore, the requirements of 35 USC § 101 are met as applied to pending Claims 14, 19-21, 23, 25 and 26, as amended.

Rejection of Claims 14, 15, 19, 25 and 26 Under 35 USC § 102(b)

Claims 14, 15, 19, 25 and 26 are rejected under 35 USC § 102(b) as being anticipated by Ross, J.A., *et al., Advances in Peritoneal Dialysis 14*:25-30 (1998) (hereinafter "Ross"). In particular, the Examiner stated that Ross discloses a human mesothelial cell population wherein all cells co-express CD49c and CD90, do not express bone cell sialoprotein, express telomerase since they are capable of proliferating and are capable of expressing IL-6.

Applicants' claimed invention, as set forth in pending amended Claims 14, 19, 25 and 26, is an isolated cell population derived from bone marrow, wherein greater than about 91% of cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours.

Ross describes a primary cell culture of human peritoneal mesothelial cells, obtained from biopsies of human omentum, which express, to varying percentages, CD49c and CD90. Ross states on page 25, that human mesothelial cells are "capable" of producing IL-6 in response to an appropriate stimulus.

There is no express or inherent teaching in Ross of an isolated cell population derived from bone marrow, wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. Therefore, Ross does not disclose the subject matter of Applicants' claimed invention, as amended. Thus, the subject matter of Claims 14, 19, 25 and 26, as amended, meets the requirements of 35 USC § 102(b) in view of Ross.

Rejection of Claims 14-20 and 22-26 Under 35 USC § 102(e) and (a)

Claims 14-20 and 22-26 are rejected under 35 USC § 102(e) as being anticipated by U.S. Patent Application No.: 2002/0168765 A1, by Prockop, D.J., *et al.,* (hereinafter "Prockop") or under 35 USC § 102 (a) as being anticipated by WO 01/34167 by Prockop, D.J., *et al.*, (hereinafter "Prockop II") in light of evidence by Cooper, L.F., *et al.*, *J. Dent. Res. 80*:314-320 (2001) (hereinafter "Cooper") and U.S. Patent No.: 5,837,539, issued to Caplan, A.I., *et al.*, (hereinafter "Caplan"). The Examiner stated that Prockop and Prockop II contain the same subject matter and, for simplicity, Prockop II formed the basis of the rejection. In particular, the Examiner stated that Prockop II discloses a cell population derived from human bone marrow

which co-express CD90 and CD49; and do not express CD34 and CD45. The Examiner further stated that the cell population of Prockop II has a doubling time of less than about 48 hours, has the potential to differentiate into pre-selected phenotype and could reasonably be expected to be capable of producing cytokines. In addition, the Examiner stated that Cooper teaches an undifferentiated mesenchymal stem cell derived from bone marrow which does not express bone sialoprotein and is relied upon to support the inherent lack of bone sialoprotein in mesenchymal stem cell populations. The Examiner stated that Caplan is relied upon to show that human mesenchymal stem cells derived from bone marrow inherently express IL-6 and the adhesion cell surface marker CD49c.

Prockop and Prockop II describe a heterogenous population of cells from bone marrow stromal cells (MSC) which express, depending upon the morphology and phase of growth of the cell, certain cell surface markers as shown in Table 3 on page 50 of Prockop II, Figure 26 of Prockop II and Table I on page 5 of Prockop. Prockop and Prockop II differentiate between the epitope profiles of cells derived from bone marrow stromal cells based on a characterization of the cells as small, rapidly self-renewing stem cells (RS-1 and RS-2 cells) and large, mature cells (mMSCs cells). As shown in Figure 26 and Table 3 of Prockop II and Table I of Prockop, only mMSCs are positive for CD90. Prockop II describes RS-1 cells as "dim" for CD90 and RS-2 cells as negative for CD90. Prockop describes RS-1 cells as "+/-," RS-2 cells as "-" and mMSC cells as "+" for CD90.

As described by Prockop on page 1, paragraphs 9 and 10, human MSCs are a hetergenous population of cells (RS and mMSC cells), which may be distinguished based on the cell surface epitopes.

> [0009] The present invention extends the observation that single-cell derived colonies of human MSCs are heterogeneous in that they contain at least two different types of cells: Small and rapidly self-renewing stem cells (RS cells) and large, more mature cells (mMSCs). It is demonstrated herein that RS cells have a greater potential for multilineage differentiation than mMSCs and a series of expressed proteins has been identified that can be used to distinguish the two cell types such that subsets of RS cells can now be identified and therefore can be isolated for use in therapy.

> [0010] The invention relates to the discovery that a population of small and rapidly self-renewing bone marrow stem cells (RS) may be further subdivided and characterized and differentiated from mMSCs on the basis of the unique expression of selected polypeptides when compared with a population of the more large, more mature marrow stromal stem cells (mMSC). RS cells the cells have been characterized a posteriori based on their varying capacities to differentiate. According to the methods of the present invention, these RS cells and MSCs may be distinguished by their protein expression profiles and a series of surface markers (epitopes) have been discovered which can be used to isolate the earliest progenitor cell of the population of marrow stem cells being studied.

(Emphasis added).

In addition, Prockop II states on page 13, lines 5-14, in reference to Figure 23, that the mMSC cells replicate poorly, may arise from RS-2 cells, but could arise from RS-1 cells and that during cell growth the cell population includes RS-1, RS-2 and mMSC cells.

> Figure 23 is a diagram of a proposed scheme for the precursor-product relationships of cells in cultures of MSCs. Large mMSCs replicate poorly (2, 41). Therefore, RS-2 cells that appear during the lag phase must arise from RS-1 cells. During the early log phase of growth, RS-2 cells decline in number as mMSCs appear in large numbers. Therefore, RS-2 cells are probably precursors of mMSCs. However, the data do not exclude the possibility that RS-2 cells rapidly generate RS-1 cells, which give rise to mMSCs (dashed-arrow). Also, the earliest mMSCs can continue to replicate. During the late log phase, RS-2 cells decline in number and the RS-1 sub-population expands. Therefore, RS-2 cells can recycle into RS-1 cells.

Further, as described by Prockop II on page 48, line 24 through page 49, line 1, the population of cells of Prockop II vary in the number of RS-1, RS-2 and mMSC cells depending upon the stage of growth.

> RS-2 cells first appeared and expanded during the lag period, as indicated in Figure 20. During the log phase of growth, the RS-2 cells decreased in number and the mMSCs rapidly

> expand. During the late log phase, the RS-2 cells disappeared and the RS-1 cells expanded.
>
> ...
>
> The data in Figures 19 and 20 indicate that the earliest progenitors in the cultures are RS-1 and RS-2 cells. Therefore, the number of RS-1 and RS-2 cells in any sample of MSCs should reflect the number of cells that generate single-cell derived colonies in CFU assays. As indicated in Figure 21, an approximately linear relationship was observed between the number of RS cells and CFU values obtained for a series of samples ($r^2 = 0.95$; p value = 0.001).

Thus, the growth kinetics and population doublings described by Prockop, in particular, Figure 22 of Prockop II, would require the presence of RS cells in a pool of marrow stem cells and could not occur in the presence of only mMSC cells in the absence of RS cells. Therefore, the cells with population doubling times depicted in Figure 22 of Prockop II could not include a large population (e.g., 91% of the cell population) of large, mature mMSC cells, which are the cells described by Prockop and Prockop II as CD90 positive.

Applicants' claimed invention, as amended, is directed to an isolated cell population derived from bone marrow wherein greater than 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. Since the RS cells of Prockop and Prockop II are "dim" or negative for CD90 as shown in Figure 22 and Table 3 of Prockop II, and Table I of Prockop, Prockop and Prockop II do not expressly or inherently teach the isolated cell population of Applicants' claimed invention. Therefore, the subject matter of pending Claims 14, 19, 20, 23, 25 and 26, as amended, meet the requirements of 35 USC § 102(e) and (a) in view of Prockop and Prockop II, in light of the evidence of Cooper and Caplan.

Rejection of Claims 14-20 and 22-26 Under 35 USC § 102(a)

Claims 14-20 and 22-26 are rejected under 35 USC § 102(a) as being anticipated by WO 01/11011 by Furcht, L.T., *et al.*, (hereinafter "Furcht") in light of Cooper and Caplan. The Examiner stated that Furcht discloses a population of multipotent adult stem cells derived from

bone marrow mononuclear cells which co-express CD90 and CD49c and do not express CD34 and CD45. The Examiner further stated the cell population described by Furcht would reasonably be expected to not express bone sialoprotein, and do have a doubling rate of 36-48 hours, have telomerase activity, have the potential to differentiate into preselected phenotypes and to produce cytokines. In addition, the Examiner stated that Cooper describes inherent properties of cells derived from bone marrow mesenchymal stem cell populations which do not express bone sialoprotein. The Examiner also stated that Caplan demonstrates that human mesenchymal stem cells derived from bone marrow inherently express several cell surface markers including CD49c and IL-6.

On page 24, lines 20-22 and as shown in Figure 2, Furcht describes a population of cells derived from bone marrow which have a cell doubling time of 36-48 hours for the initial 20-30 cell doublings and afterward a cell doubling time of 60-72 hours. Furcht also describes a cell population derived from bone marrow with a cell doubling time of 48-60 hours which stain positive with antibodies directed to, *inter alia*, CD90 (page 73, lines 13-18).

There is no disclosure or suggestion in Furcht of an isolated cell population derived from bone marrow which has a population doubling time of less than about 30 hours and wherein greater than about 91% of the cells in the isolated cell population co-express CD49c and CD90, which is the subject matter of Applicants' claimed invention, as amended. Therefore, Furcht does not disclose Applicants' claimed invention, as set forth in amended pending Claims 14, 19, 20, 23, 25 and 26.

The subject matter of Applicants' claimed invention, as set forth in pending Claims 14, 19, 20, 23, 25 and 26, as amended, meet the requirements of 35 USC § 102(a) in view of Furcht in light of the evidence of Cooper and Caplan.

Rejection of Claims 14-26 under 35 USC § 103

Claims 14-26 are rejected under 35 USC § 103(a) as being unpatentable over Prockop, Prockop II and Furcht in light of evidence by Cooper and Caplan taken with Bos, C., *et al., Cell Tissue Res. 293*:463-470 (1998) (hereinafter "Bos") and Gartel, A.L., *et al., Experimental Cell Res. 246*: 280-289 (1999) (hereinafter "Gartel"). The Examiner stated that although Prockop and Prockop II do not teach expression of MCP-1, Furcht teaches expression of MCP-1 by a cell

population derived from bone marrow. The Examiner further stated that although Cooper and Caplan do not disclose all cell adhesion epitopes, for example, CD90, stem cells or doubling times for stem cells, Cooper and Caplan teach mesenchymal stem cell populations derived from human bone marrow cells as described in Applicants' invention. In addition, the Examiner stated that Bos teaches human mesenchymal stem cells derived from bone marrow which express p21 and Gartel demonstrates that expression of p21 and p53 are related events in cell cycle progression. The Examiner further stated on page 10 of the Office Action:

> [A]lthough the referenced cell populations of ... [Prockop, Prockop II, Furcht, Cooper and Caplan] might not be identical to the presently claimed cell population with regard to the expression and the amounts of p21 and p53 transcripts, the difference between that which are disclosed and that which are claimed are considered to be so slight that the reference cell populations are likely to possess the same characteristics of the claimed cell population.

As discussed above, Applicants' claimed invention, as amended, is directed to an isolated cell population derived from bone marrow, wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. Applicants' claimed cell population, as amended, is believed to be a consequence of culturing cells derived from bone marrow under low oxygen conditions, such as 5% oxygen, as described in the specification on, for example, page 2, lines 25-29; page 4, lines 7-9; page 8, lines 10-12; page 11, line 24 through page 12, line 1; page 14, lines 20-23; page 15, lines 6-8; and page 20, lines 4-6. The low oxygen conditions are defined in the specification on page 14, lines 21-23 as:

> "Low oxygen conditions," as used herein, refers to a concentration (e.g., percent of oxygen based on volume, weight or molarity) which is less than atmospheric oxygen.

As shown in the twelfth edition of Hawley's Condensed Chemical Dictionary (1993) (Exhibit A), atmospheric oxygen or air is composed of about 23% oxygen (by weight), or about 21% oxygen (by volume). Reference to low oxygen conditions employed to obtain Applicants'

claimed cell population is set forth, for example, on page 25, lines 24-27; page 27, lines 20-23; page 29, lines 9-15; and page 30, lines 5-10 of the specification as "5% carbon dioxide, 5% oxygen, and 90% nitrogen/air" means the air component of the culture condition is 5% carbon dioxide, 5% oxygen and 90% nitrogen. Percentages refer to volume percents. Thus, the concentration of oxygen employed by Applicants is 5% oxygen (by volume).

As discussed above, there is no disclosure or suggestion in Prockop or Prockop II of an isolated cell population derived from bone marrow wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the cell population has a doubling rate of less than about 30 hours. The cells of Prockop and Prockop II vary significantly in growth rate and expression of CD90. In addition, there is no disclosure or suggestion in Prockop or Prockop II of culturing cells derived from bone marrow under conditions of low oxygen, in particular, at an oxygen concentration substantially less than that of atmospheric oxygen, such as employed by Applicants, to generate Applicants' claimed isolated cell population. As discussed on page 4, paragraph 56 of Prockop; page 29, lines 22-23 and page 46, lines 5-6 of Prockop II, cells were cultured in "5% humidified CO_2." The balance of air used to culture the cells of Prockop and Prockop II appears to be atmospheric oxygen. Atmospheric oxygen is about 21% oxygen (by volume), as shown in Exhibit A.

As also discussed above, Furcht does not disclose or suggest an isolated cell population derived from bone marrow, wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. As discussed above, Furcht describes a population of cells with a doubling rate of 36-48 hours, 48-60 hours and 60-72 hours. On page 22, lines 28 through page 23, line 1, Furcht references Muschler, G.F., *et al., J. Bone Joint Surg. Am. 79*:1699-1709 (1997) (hereinafter "Muschler") and Batinic, D., *et al., Bone Marrow Transplant. 6*:103-107 (1990) (hereinafter "Batinic") as standard means to derive bone marrow mononuclear cells from bone marrow aspirates. Muschler and Batinic are attached to this Reply as Exhibits B and C. As noted on page 1701 of Muschler, bone marrow cells were cultured in 5% CO_2, which suggest a balance of air which contains about 21% oxygen (by volume). As shown in Exhibit C, Baltinic does not culture bone marrow cells.

Furcht does not remedy the deficiencies of Prockop or Prockop II. Specifically, there is no disclosure or suggestion in Prockop, Prockop II or Furcht, taken either separately, or in any combination, of an isolated cell population having a doubling rate of less than about 30 hours wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90. Further, there is no disclosure or suggestion in Prockop, Prockop II, or Furcht, taken either separately or in any combination, of obtaining a cell population from bone marrow by culturing the bone marrow cells under conditions of low oxygen, which is a condition employed by Applicants to obtain Applicants' claimed invention, as set forth in pending Claims 14, 19-21, 23, 25 and 26. Therefore, neither Prockop, Prockop II or Furcht, taken either separately or in any combination, disclose or suggest Applicants' claimed invention, as amended.

Cooper describes the expression of osteogenic markers including bone sialoprotein, during *in vivo* osteogenesis of bone marrow stem cell-based implants. The purpose of Cooper is to study osteogenesis. Cooper does not teach or suggest an isolated cell population derived from bone marrow, wherein greater than 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours, which is the subject matter of Applicants' claimed invention, as amended.

Cooper does not remedy the deficiencies of Prockop, Prockop II or Furcht. Specifically, there is no disclosure or suggestion in Prockop, Prockop II, Furcht or Cooper, taken either separately or in any combination, of a cell population derived from bone marrow, wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. Therefore, Applicants' claimed invention, as amended, is not obvious in view of any of these references, taken either separation or in any combination.

Caplan describes the isolation and purification of mesenchymal stem cells, including mesenchymal stem cells obtained from bone marrow. As described in column 21, lines 5-9 of Caplan, bone marrow cells were cultured in a humidified atmosphere containing 95% air and 5% CO_2. As noted in Exhibit A, air consists of about 21% oxygen (by volume) atmospheric oxygen.

Caplan does not remedy the deficiencies of Prockop, Prockop II, Furcht or Cooper. In particular, there is no disclosure or suggestion in Prockop, Prockop II, Furcht, Cooper or Caplan, taken either separately on in any combination, of the cell population in which greater than about

91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. In addition, there is no disclosure or suggestion in Prockop, Prockop II, Furcht, Cooper, or Caplan, taken either separately or in any combination, of culturing cells derived from bone marrow under conditions of low oxygen, which is an oxygen concentration less than atmospheric oxygen, as described in the specification of Applicants' invention, for example, on page 14, lines 21-23. Therefore, Prockop, Prockop II, Furcht, Cooper or Caplan, taken either separately or in combination, do not disclose or suggest Applicants' claimed invention, as amended.

Bos is directed to assessing the role of apoptosis and p21 and p27 protein expression in skeletal development. Bos describes a decrease in the levels of p21 and p27 with an increase in the occurrence of apoptosis. On page 467, Bos speculates that when bone marrow stem cells are cultured at low density, levels of p27 and p21 are reduced, which causes cells to engage prematurely in DNA replication, thereby leading to apoptosis. On page 464, Bos describes the culture of human marrow stem cells from bone marrow aspirates as performed employing methods previously described by Bos, C., *et al., Human Cell 10*:45-50 (1997) (hereinafter "Bos II") and Bruder, S.P., *et al., J. Cell Biochem., 64*:278-294 (1997) (hereinafter "Bruder"). Bos II and Bruder are attached to this Reply as Exhibits D and E respectively. Bos II, in turn, on page 46, in the section entitled "Preparation of human mesenchymal stem cell," references Haynesworth, S.E., *et al., Bone 13*:69-80 (1992) (hereinafter "Haynesworth") and Caplan, A.I., *et al.*, In: Lanza, R.P., et al. (eds), pp. 603-618 (1997) (hereinafter "Caplan II") in the preparation of mesenchymal stem cells. Haynesworth and Caplan II are attached to this Reply as Exhibits F and G, respectively.

As noted on page 70 of Haynesworth, in the section entitled "Culture and passage of marrow-derived mesenchymal cells," cells are cultured in 95% air and 5% CO_2. No reference to specific culture conditions is made in Caplan II.

As noted in Bruder, on page 280 of Exhibit E, human marrow stem cells were cultured in "a humidified atmosphere containing 5% CO_2," which is a condition of employing atmospheric oxygen, that, as discussed above and as shown in Exhibit A, is about 21% oxygen (by volume). Thus, the culture conditions of Bos II and Bruder consist of culturing human bone marrow stem

cells in atomospheric oxygen, not low oxygen concentrations, as employed by Applicants, to generate Applicants' claimed cell population.

As with Prockop, Prockop II, Furcht, Cooper and Caplan, there is no disclosure or suggestion in Bos of the subject matter of Applicants' cell populations, as set forth in amended Claims 14, 19-21, 23, 25 and 26, which includes a cell population derived from bone marrow, wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours. Thus, Bos does not remedy the deficiency of Prockop, Prockop II, Furcht, Cooper or Caplan, since none of these references, taken either separately, or in any combination, disclose or suggest a cell population as set forth in Applicants' claimed invention, as amended. In particular, none of these references disclose a cell population derived from bone marrow wherein greater than 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling rate of less than about 30 hours.

Gartel describes the transcriptional regulation of the p21 gene. On page 281, Gartel states that two p53-responsive elements have been identified in p21 promoter sequences. However, Gartel further states on page 281 that "Expression of p21 appeared normal in embryos and most tissues of mice lacking a functional p53 gene." In addition, Gartel states, on page 281, that "p53 is not required for induction of p21 transcription during development and in most tissues of the adult mouse." Beginning on page 283, Gartel describes a number of p53-independent regulations of p21 gene expression. Thus, according to Gartel the p21 gene can be regulated in the absence of p53.

As with Prockop, Prockop II, Furcht, Cooper, Caplan and Bos, there is no disclosure or suggestion in Gartel of the subject matter of Applicants' claimed cell population, as amended. Specifically, there is no mention of any cell population in Gartel which co-express CD49c and CD90 and has a population doubling rate of less than about 30 hours. Gartel does not remedy the deficiency of Prockop, Prockop II, Furcht, Cooper, Caplan and Bos, since none of these references, taken either separately or in any combination, disclose or suggest a cell population derived from bone marrow cells, wherein greater than about 91% of the cells of the isolated cell population co-express CD49c and CD90 and wherein the isolated cell population has a doubling

rate of at least about 30 hours. Therefore, Gartel does not remedy the deficiency of any of the references cited by the Examiner.

Taken separately or in any combination, Applicants' claimed invention, as amended, meets the requirements of 35 USC § 103(a) in view of Prockop, Prockop II and Furcht in light of Cooper and Caplan taken with Bos and Gartel, either separately or in combination.

SUMMARY AND CONCLUSIONS

Applicants have amended Claims 14, 19-21, 23, 25 and 26. Applicants have canceled Claims 15-18, 22 and 24. As amended, Claim 14 meets the requirements of 35 USC § 112, second paragraph. As amended Claims 14, 19-21, 23, 25 and 26 meet the requirements of 35 USC § 112, second paragraph and 35 USC § 101. The subject matter of amended Claims 14, 19, 25 and 26 meets the requirements of 35 USC § 102(b) in view of Ross. Further, amended Claims 14, 19, 20, 23, 25 and 26 meet the requirements of 35 USC §§ 102(e) and (a) in view of Prockop and Prockop II, in light of Cooper and Caplan. In addition, amended Claims 14, 19, 20, 23, 25 and 26 meet the requirements of 35 USC § 102(a) in view of Furcht, in light of Cooper and Caplan. In addition, as amended, Claims 14, 19-21, 23, 25 and 26 meet the requirements of 35 USC § 103(a), in view of Prockop, Prockop II and Furcht in light of Cooper and Caplan, taken with Bos and Gartel. Therefore, Applicants respectfully request reconsideration and allowance of the claims under consideration.

If the Examiner feels a telephone conference would expedite prosecution of this application, she is invited to call Applicants' undersigned attorney.

Respectfully submitted,

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Dated: December 22, 2003